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54 Mycoplasma Bovis Combination Vaccines for Cattle

57 Mycoplasma bovis combination vaccines (MbK vaccines), characterized in that said vaccines contain
- 10-90 parts by volume of an inactivated mixture of pathogen components comprising
1-9 parts by volume of inactivated bacterial components and
9-1 parts by volume of inactivated mycoplasmal components,
with both components containing customary inactivation agents.
- 25-90 parts by volume of customary oil adjuvants or 5-10 parts by volume of customary mineral adjuvants, and
- up to 0.45 parts by volume of customary preservatives.

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Mycoplasma Bovis Combination Vaccines for Cattle

Mycoplasma bovis combination vaccines (MbK vaccines) are used to fight infectious diseases of the respiratory organs in cattle, in particular in calves.

Infectious diseases of the respiratory organs in cattle are caused by mixed infections from viruses, mycoplasmas, and certain types of bacteria. Infections of this type of the respiratory organs in cattle often lead to disease and death. Primarily calves are affected, in the period leading up to and during the first phase of fattening.

Pathogens described for these infections of the respiratory organs include several types of viruses, such as infectious bovine rhinotracheitis (IBR)—the principal virus—and mucosal disease (MD), bovine respiratory syncytial virus (BRSV), and parainfluenza virus type 3 (PI-3-V), and several types of bacteria such as Mannheimia (Pasteurella) haemolytica, Pasteurella multocida, Klebsiella pneumoniae, and Moraxella bovis, in addition to chlamydias (Chlamydia psitaci) and mycoplasmas such as Mycoplasma bovis (M.b.) and Mycoplasma bovirhinis (M.b.r.). Mixed infections are usually involved, with varying participation of the referenced types of pathogens.

Furthermore, it is known that preventive vaccinations can be effective against these infections. Many commercial vaccines exist worldwide to fight the virus components of these mixed infections. In the Federal Republic of Germany, there is only one vaccine against Mannheimia (Pasteurella) haemolytica infection (Pastobov, from Merial) for immunoprophylaxis, to fight the bacterial components. No vaccine is known against the mycoplasmal component in mixed infections.

Literature reports on experimental studies, such as Schimmel, D., "Infektionskrankheiten der Haustiere" [Infectious Diseases of Domestic Animals], Mykoplasmen-Infektionen [Mycoplasmal Infections], Part II, p. 417; Pfützner, H. and Sachse, K.: Rev. sci. Off. int. Epiz. 15, 1477-1494 (1996) indicate that the clinical relevance of mycoplasmas in mixed infections of the respiratory organs in cattle is considered low. Following vaccine tests with inactivated mycoplasmas, it was determined that protection against infection was insufficient, and that adequate immunization against the mycoplasmal infection was not achieved. The results of these studies have led medical experts to conclude that no effective immunoprophylaxis against mycoplasmal infection can be developed in the form of a vaccine.

In contrast to the aforementioned views prevalent in Austria and Germany, the patent holder's own diagnostic tests have led to the findings that mycoplasmas (M.b. and M.b.r.) are primary participants as pathogens causing infections of the respiratory organs in cattle. This is also true for bacterial pathogens, principally Pasteurella multocida and Mannheimia (Pasteurella) haemolytica, which play a part in causing diseases of the respiratory organs leading to death.

The object of the invention, therefore, is to find vaccines that assure effective immunoprophylaxis against diseases of the respiratory organs in cattle which result from infections with mycoplasmas and mixed infections with other bacterial pathogens.

It has been found that combination vaccines—which contain a mixture of freshly inactivated mycoplasmal cattle pathogens (mycoplasmal components) isolated from cattle, and pneumotropic types of bacteria (bacterial components)—surprisingly show very good preventive protection against mixed infections of the respiratory organs in cattle, especially in calves.

The mycoplasmal component preferably contains Mycoplasma bovis (M.b.) and/or Mycoplasma bovirhinis (M.b.r.).
The bacterial component contains various types of bacteria, individually or in combination. The following bacteria are preferably constituents of the bacterial component: Mannheimia (Pasteurella) haemolytica, Pasteurella multocida, Moraxella bovis, and Klebsiella pneumoniae.

The novel MbK vaccines, according to the invention, contain the components according to Claims 1 through 11 and are used according to Claim 12.
First, the active components of the MbK vaccines—the mycoplasmas and bacteria—were obtained from nasal swabs and/or organ sections and/or lung sections, altered by inflammation, from cattle affected by mixed infections. To this end, the samples taken from the aforementioned parts of cattle for the isolation of mycoplasmas were cultivated in Hayflick medium,

filtered, passaged, and cultured on plates for testing and reisolation. The types of mycoplasma thus isolated were propagated under CO in three cultivation steps. The concentration of the pathogen was then determined by turbidity measurement and live bacterial count. The isolated mycoplasmal cultures obtained were inactivated, with cooling, by the addition of up to 0.1 parts by volume of a customary inactivation agent, preferably β-propiolactone.

To isolate bacteria, samples taken from the aforementioned parts of cattle were placed on specialized nutrient plates. After incubation, individual types of bacteria were reisolated, identified, cultivated in specialized media, and cultured in 3-4 cultivation steps and, after reaching the stationary growth phase, were inactivated by heating or by addition of up to 0.2 parts by volume of a customary inactivation agent, preferably approximately 10% formaldehyde solution.

1-9 parts by volume of the inactivated pathogen component containing mycoplasmas are mixed with 9-1 parts by volume of the inactivated pathogen component containing bacteria. The mixture preferably contains 5 parts by volume each of the inactivated mycoplasmal cultures and the inactivated bacterial cultures. Customary mineral or oil adjuvants are added to this mixture, as well as customary preservatives.

An MbK vaccine based on oil adjuvants contained 10-90 parts by volume of the inactivated mixture of pathogen components, 25-90 parts by volume of a conventional oil adjuvant, preferably mineral oil, and up to 0.45 parts by volume of a customary preservative.

An MbK vaccine based on mineral adjuvants contained 90-95 parts by volume of the inactivated mixture of pathogen components, 5-10 parts by volume of a conventional mineral adjuvant, preferably aluminum compounds, in particular aluminum sulfate and/or aluminum hydroxide of DAB (Deutsches Arzneimittelbuch [German Pharmacopeia]) purity, and up to 0.45 parts by volume of a customary preservative.

As preservative, preferably 0.25-0.45 parts by volume phenol or up to 0.01 parts by volume thiomersal of DAB purity are contained in the MbK vaccines.

When the novel MbK vaccines were used to prevent diseases of the respiratory organs in cattle caused by mixed infections, 75-80% of the animals treated were protected from infection.

The following examples embody several preferred constituents of the novel MbK vaccines.

Example 1:

For the preparation of approximately 1 L MbK vaccine, 380 mL mycoplasmal culture was inactivated with 0.38 mL beta-propiolactone. At the same time, 380 mL bacterial culture or mixed bacterial culture was inactivated with 7.6 mL of a 10% formaldehyde solution. An emulsion was produced from this aqueous mixture by the addition of 200 mL oil components. The emulsion was preserved with 5 mL of a 2% thiomersal solution.

Example 2:

For the preparation of approximately 1 L MbK vaccine, 65 mL mycoplasmal culture was inactivated with 0.07 mL beta-propiolactone. At the same time, 650 mL bacterial culture or mixed bacterial culture was inactivated with 13 mL of a 10% formaldehyde solution. An emulsion was produced from this aqueous mixture by the addition of 300 mL oil components. The emulsion was preserved with 5 mL of a 2% thiomersal solution.

Example 3:

For the preparation of approximately 1 L MbK vaccine, 650 mL mycoplasmal culture was inactivated with 0.65 mL beta-propiolactone. At the same time, 65 mL bacterial culture or mixed bacterial culture was inactivated with 1.3 mL of a 10% formaldehyde solution. An emulsion was produced from this aqueous mixture by the addition of 250 mL oil components. The emulsion was preserved with 5 mL of a 2% thiomersal solution.

Claims:

1. Mycoplasma bovis combination vaccines (MbK vaccines), characterized in that said vaccines contain
   - 10-90 parts by volume of an inactivated mixture of pathogen components comprising
     1-9 parts by volume of inactivated bacterial components and
     9-1 parts by volume of inactivated mycoplasmal components, with both components containing customary inactivation agents.
   - 25-90 parts by volume of customary oil adjuvants or 5-10 parts by volume of customary mineral adjuvants, and
   - up to 0.45 parts by volume of customary preservatives.

2. MbK vaccines according to Claim 1, characterized in that the mixture of pathogen components preferably contains 5 parts by volume each of bacterial and mycoplasmal components.

3. MbK vaccines according to Claims 1 and 2, characterized in that the bacteria and mycoplasmas for the pathogen components of the MbK vaccines are isolated from organ sections and/or lung sections altered by inflammation and/or nasal swabs from cattle affected by mixed infections.

4. MbK vaccines according to Claims 1 through 3, characterized in that the bacterial component contains various types of bacteria, preferably Mannheimia (Pasteurella) haemolytica, Pasteurella multocida, Moraxella bovis, and Klebsiella pneumoniae, individually or in combination.

5. MbK vaccines according to Claims 1 through 4, characterized in that the bacterial cultures and the mixed bacterial cultures are inactivated by heating.

6. MbK vaccines according to Claims 1 through 4, characterized in that the bacterial cultures and the mixed bacterial cultures preferably contain up to 0.2 parts by volume of a 10% formaldehyde solution as inactivation agent.

7. MbK vaccines according to one of Claims 1 through 3, characterized in that the mycoplasmal component preferably contains the mycoplasmas Mycoplasma bovis (M.b.) and/or Mycoplasma bovirhinis (M.b.r.).

8. MbK vaccines according to Claims 1 through 3 and 7, characterized in that the mycoplasmal cultures preferably contain up to 0.1 parts by volume β-propiolactone.

9. MbK vaccines according to one of Claims 1, characterized in that said vaccines contain mineral oils as customary oil adjuvants.

10. MbK vaccines according to one of Claims 1, characterized in that said vaccines preferably contain aluminum compounds, in particular aluminum sulfate and/or aluminum hydroxide, as customary mineral adjuvants.

11. MbK vaccines according to one of Claims 1, characterized in that said vaccines preferably contain 0.25-0.45 parts by volume phenol or 0.01 parts by volume thiomersal.